Vegetation Responses to *Pinus radiata* (D. Don) Invasion: A Multivariate Analysis Using Principal Response Curves

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Radiata pine (*Pinus radiata* D. Don) has been introduced to many new regions outside its native range as a plantation species. Plantations are frequently located adjacent to native vegetation. This proximity allows not only pine wildings, but also large amounts of non-native leaf litter, to enter the surrounding natural vegetation. Our aim in the present study was to assess the composition of plant communities in vegetation surrounding plantations in relation to proximity to pine plantations. Using multivariate Principal Response Curves (PRC) analysis, we show significant differences in the composition of native vegetation between transects adjacent to and not adjacent to pine plantations. Species-level analysis identified a suite of native species that were frequently found in transects adjacent to pine plantations, and a second suite of native species that were reduced in abundance in transects next to pine plantations. This second group of species should be the focus of future conservation work, since they appear to be sensitive to disturbance wrought by pine plantations. We show that the ability of PRC analysis to reveal both community-level and species-level responses to disturbance brought about by exotic species can lead to the generation of testable hypotheses bridging species and community ecology.

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INTRODUCTION

The timber of *Pinus radiata* (D. Don) is valuable because of its numerous applications in manufacturing and construction industries (Sutton 1999). Consequently, extensive areas now support plantations globally, making *P. radiata* the most commonly grown conifer in the world (Lavery and Mead 1998). In Australia, pine plantations are commonly bordered by native vegetation (Williams and Wardle 2005), and are frequently established amongst native vegetation and/or contain areas of remnant vegetation within the plantation (Lindenmayer et al. 2002, Lemckert et al. 2005). Of particular concern is the occurrence of plantations in close proximity to areas set aside for conservation purposes. For example, to the west of Sydney, pine plantations border Blue Mountains National Park, Kanangra-Boyd N.P., and Jenolan Caves Karst Conservation Reserve. The close proximity of plantations to native vegetation is problematic because *P. radiata* is highly invasive and can readily escape and establish in native vegetation, beyond the boundaries of the plantation (Richardson and Higgins 1998). For instance, prior to removal of the Jounama Pine Plantation (southern NSW, Australia), an estimated 16 000 *Pinus* spp. individuals were growing in 24 000 ha of adjacent native vegetation in Kosciusko National Park (Leaver 1983).

The establishment of *P. radiata* within remnant native vegetation has been linked to the displacement of native plant species (Richardson et al. 1994, Richardson and Higgins 1998, Holmes et al. 2000, Morgan et al. 2000). When pines become well established, as in plantations, the richness of bryophytes, vertebrates and invertebrates is reduced relative to undisturbed native forest (Bonham et al. 2002, Lindenmayer and Hobbs 2004, Parris and Lindenmayer 2004, Pharo et al. 2004). In this study, we compare patterns of plant distribution between patches of remnant vegetation that are adjacent to, and not adjacent to pine plantations. Those areas adjacent to pine plantations are not only subject to invasion from pine wildlings, but also receive a large amount of pine litter (needles, pollen cones) from the adjacent plantation (Baker 2004). This material may smother ground-covering plants, and may alter soil chemistry and further facilitate change in the composition of vegetation communities. Our hypotheses are; 1) that there will be a significant difference in plant species richness and abundance between areas adjacent to and not adjacent to *P. radiata* plantations, and 2) that these differences will decrease with distance from the plantation as the number of wildings and pine litter also decreases.

To test these hypotheses, we use the multivariate method of Principal Response Curves (PRC). The PRC technique has been used widely in ecotoxicology (e.g. Cuppen et al. 2000, Hose et al. 2003, Belanger et al. 2004) and is gaining some recognition as a tool for biomonitoring studies in aquatic ecology (e.g. Leonard et al. 1999, Pardal et al. 2004). PRC analysis has only very recently been used in vegetation ecology (e.g. Pakeman et al. 2003, Heegaard and Vandvik 2004, Vandvik et al. 2005). The utility of PRC analysis is not yet widely recognised for ecological studies. Our investigation of the impact of pine plantations on the composition of remnant vegetation provided us with an ideal opportunity to demonstrate the applicability of the PRC technique for ecological studies.

METHODS

Study Location and Design

This study was conducted in the Jenolan Karst Conservation Reserve (33° 49'S, 150° 02'E), in southeast Australia, from April to August 2004 (Baker 2004). The native vegetation of the study area is *Eucalyptus* spp. dominated woodland, with an understorey dominated by herbs, grasses and the occasional larger shrub species (e.g. *Acacia longifolia* and *Exocarpus strictus*).

A narrow trail (~5 m wide) separates the Jenolan Karst Conservation Reserve from adjoining areas of mature *P. radiata* plantation and remnant forest. This allowed us to place sampling transects in woodland areas adjacent to *P. radiata* plantations (nominally 'disturbed' areas) and in woodland areas adjacent to remnant vegetation (nominally 'undisturbed' areas). Nine replicate transects were randomly placed in disturbed and nine in undisturbed areas. The areas were all similar in altitude, aspect, slope, fire history, topography and soil type, but differed in being either adjacent to or not adjacent to pine plantations. Consequently, we have confidence in attributing any observed differences in vegetation composition between sites to the disturbance, having minimised confounding inter-site differences. Transects were laid parallel to each other and perpendicular to the trail, extending 50 m into the reserve. Sampling was conducted at 10 m intervals along the transect line (i.e. 6 samples per transect), using a 2 m x 5 m quadrat. Our study focused on herbs and shrubs as these are most likely to be affected by pine invasions, hence we ignored the canopy species (*Eucalyptus* spp.) as we considered them to have been established prior to the pine plantation and thus minimally affected. All vascular plants within the quadrat were identified and the percent canopy cover of each species within the quadrat was recorded as a measure of abundance.

Data Analysis

The technique of Principal Response Curves is a novel multivariate statistical method. PRC analysis was developed for the analysis of multi-species data from experiments designed with replicated controls and treatments and, specifically, repeated temporal sampling (van den Brink and Ter Braak 1999). The PRC method focuses on differences between treatment and controls at each sampling time. It provides simplified ordination plots in which temporal gradients are presented along a horizontal, unidirectional axis. Here, we show that PRC analysis is equally applicable to transect studies with repeated sampling over spatial, rather than temporal gradients. In the following description, a simplified overview of the PRC technique is provided, and readers are referred to van den Brink and Ter Braak (1999) for full details of the method.

The PRC method is based on Redundancy Analysis (RDA) but is extended to adjust for changes in the controls over time (van den Brink and Ter Braak 1999). RDA can be considered a constrained form of Principal Components Analysis, meaning that patterns in the biological data are limited to that which can be explained by explanatory variables. Because in RDA the explanatory variables are fixed *a priori*, the total variance can be partitioned into explained and residual variances.

A major problem with traditional ordination plots is that they may be congested, and differences among treatments and controls, and temporal trajectories can be confusing, particularly when ordination plots contain repeated sampling over time or space (van den Brink and Ter Braak 1999). To avoid these problems, PRC uses explanatory variables that distinguish the controls from the treatment, and individual sampling events (times or in our case distances). Explanatory variables identifying the controls are deleted from the analysis to ensure that the treatment effects are expressed as a deviation from the control, at each distance (van den Brink and Ter Braak 1999).

We used CANOCO version 4 (Ter Braak and Smilauer 1998) to carry out the PRC analysis. Like its precursor RDA, PRC requires a linear response model (van den Brink and Ter Braak 1999). To test the suitability of a linear model, Detrended Correspondence Analysis was used to determine the gradient length (the beta diversity, or extent of species turnover) of the first axis. The gradient length of the first axis was 3.08. Gradients >4 suggest a unimodal model is needed because data are heterogeneous and many species deviate from the assumed linear response model. Gradients ≤ 3 are better suited to analyses with linear models such as PCA or RDA (Leps and Smilauer, 2003).

PRC also requires the use of the Euclidean Distance for sample (dis)similarities (van den Brink and Ter Braak 1999). Euclidean distance weights shared absences and shared presences of species equally in its assessment of similarity among samples, thus distances among samples are driven by differences in the abundance of taxa irrespective of whether those species are present or absent in either sample. This is desirable for this study because differences among samples are not greatly influenced by chance recordings of uncommon species.

Plant abundance data were log(10x+1) transformed prior to analysis and all other recommended settings were used (van den Brink and Ter Braak 1999). Because plants multiply or die, count data are naturally modelled by proportional changes, i.e. by a multiplicative model. We used a logarithmic transformation, so as to turn the multiplicative model for the counts into a linear model (van den Brink and Ter Braak 1999) and to down-weight the dominant species in the vegetation assemblages. Multiplication by the constant (10) avoided false discrepancy between zero and low abundance values (van den Brink et al. 1995).

In a PRC diagram, the horizontal axis represents the distance along transects of the experiment and the vertical axis represents the treatment effect (Canonical Coefficient C_{dt}) expressed as deviations from the control. The accompanying species weights allow an interpretation of effects at the species level. In the present study, taxa with negative species weights are expected to increase in abundance in the disturbed areas relative to the undisturbed areas, and taxa with positive species weights are expected to decrease in abundance in the disturbed areas relative to the undisturbed areas. Taxa with near zero weights either show no response or a response that is unrelated to the PRC (van den Brink and Ter Braak 1999).

The significance of the treatment regime was tested using Monte Carlo tests and permuting whole transects among disturbed and undisturbed areas. Further Monte Carlo tests were performed to test the significance of differences at each distance along transects. This was achieved by conducting Monte Carlo tests using only those data for the distance of interest (van den Brink et al. 1996). For the Monte Carlo tests, a binary coded explanatory variable was used to distinguish transects in disturbed and undisturbed areas in the analysis. The Monte Carlo permutation tests are based on an F-type statistic, and the significance level (α) was 0.05.

RESULTS

Our PRC analysis detected significant differences ($p = 0.005$) in the composition of plant communities between disturbed and undisturbed areas. Differences among treatments accounted for 10.5% of all variance, while differences among sampling distances accounted for 6.1% of all variance. The remainder was attributed to variability among replicates. The response pattern in the first PRC axis was significant ($p = 0.03$), and this axis captured a much greater proportion of the total variance explained by the treatment regime than the second axis, which was not significant ($p = 0.205$). For this reason, only the first axis of the PRC analysis is presented.

At the trail (distance = 0 m) and closest to the plantations, the disturbed and undisturbed areas differed greatly, but became more similar with increasing distance along transects into the remnant vegetation (Fig. 1). This pattern was consistent with the results of Monte Carlo tests, which detected significant ($p<0.05$) treatment effects at 0 and 10 m but not at greater distances into the remnant vegetation ($p>0.05$, Fig. 1).

The difference among disturbed and undisturbed areas was most strongly driven by differences in the abundance of *P. radiata*, which had a strongly negative species weight (Fig. 1). Native species with large negative species weights were *Lomandra longifolia, Leucopogon lanceolatus, Poranthera microphylla*, and *Cassinia aculeata*, suggesting an affinity of these species to the disturbed areas. Native species with strongly positive species weights, such as *Persoonia acuminata, Monotoca scoparia, Clematis aristata* and *Stellaria pungens* were more abundant in the undisturbed sites, demonstrating the sensitivity of these species to the disturbance associated with the penetration of pine litter.

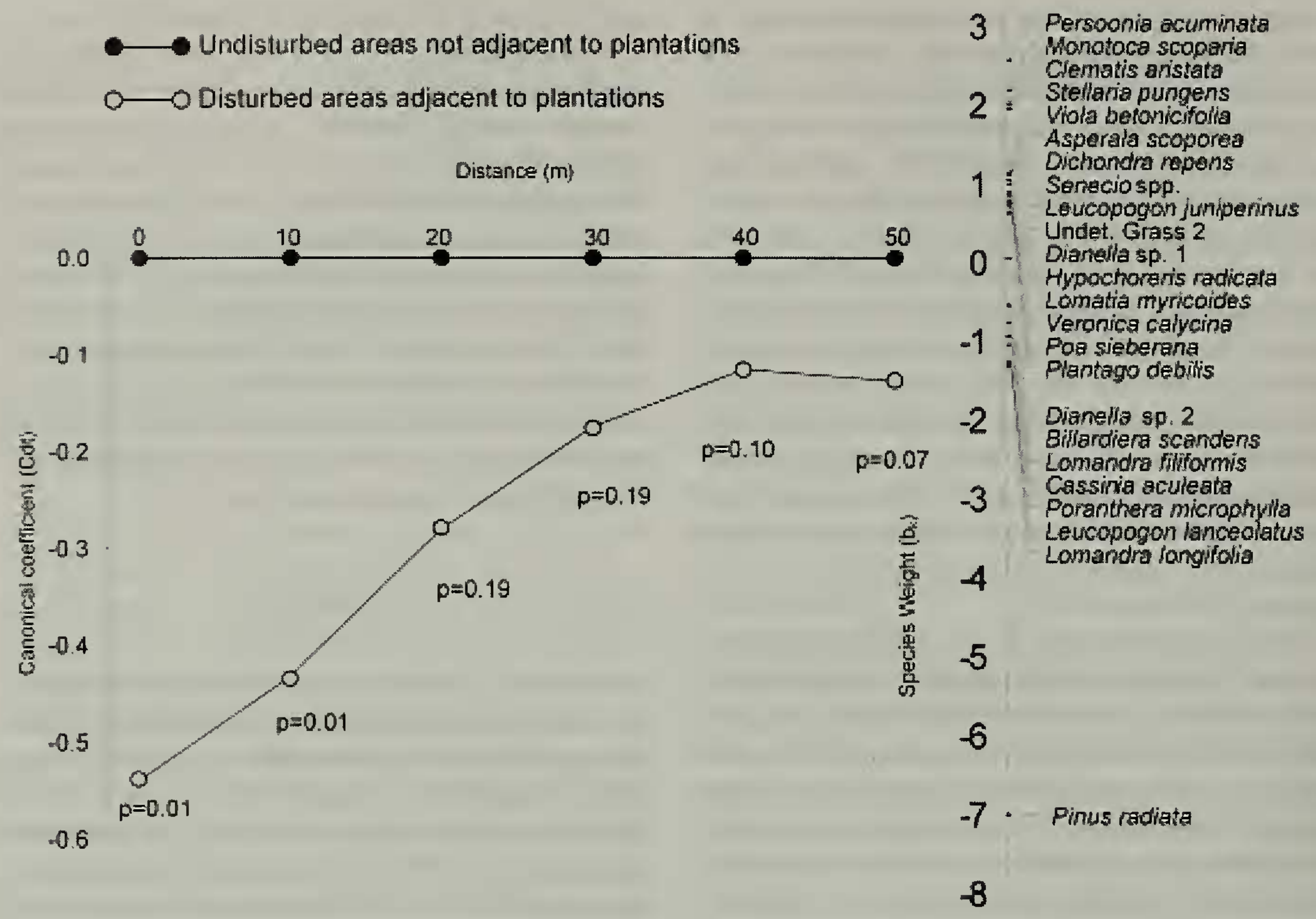

Figure 1. Principal response curve with species weights for vegetation assemblage data from vegetation transects in areas adjacent to and not adjacent to pine plantations. Species with weights between 0.5 and -0.5 have been omitted for clarity. Probability (p) values indicate the outcomes of Monte Carlo tests per sampling distance.

DISCUSSION

The significant differences in the distribution of plant species located at disturbed and undisturbed areas in this study are highly consistent with previous research in the southern hemisphere that has documented the displacement of native plant species following invasion by *P. radiata* (Richardson et al. 1994, Richardson and Higgins 1998, Holmes et al. 2000, Morgan et al. 2000). We have also shown that differences in the composition of plant communities between disturbed and undisturbed areas decrease with distance from plantations, such that there is no significant difference in plant species richness and abundance at 20 m and beyond. The increasing similarity of plant communities with increased distance from plantations is consistent with work showing an exponential decline in the mass of pine litter with increasing distance from plantations (Baker 2004).

Relative changes in the composition of native plant communities between disturbed and undisturbed areas are clearly evident and easily interpretable in the PRC, therein highlighting a significant advantage of this approach over traditional ordination techniques. The method also has the distinct advantage over other approaches in that the ordination allows a simplified interpretation of species-level patterns in the data. Thus, this method is likely to detect subtle changes that may occur in only a few species in the assemblage (Pardal et al. 2004). The PRC method is currently limited to using Euclidean distance as the (dis)similarity measure. The trade off is the ability for PRC to include a species-level analysis. Other analyses that permit a broader range of indices (e.g. similarity analysis, MDS, ANOSIM) have a limited ability to display effects on particular species (although species patterns can be shown through supplementary analyses such as SIMPER). An advantage of PRC analysis is that it integrates sample ordinations and species patterns in a single analysis (van den Brink and Ter Braak 1999).

Accompanying the increase in pine abundance, the vegetation of disturbed sites contained a greater abundance of *Lomandra longifolia, Leucopogon* *lanceolatus, Poranthera microphylla*, and *Cassinia aculeata* than at undisturbed sites. Several of these species are able to colonise disturbed habitats (B.R. Murray pers. obs.), however, there are very few herbarium records that describe whether or not these species are characteristic of disturbed areas. The exception is *Cassinia aculeata*, which is known to be a fast growing pioneer species that regenerates from seed following a disturbance (CSU herbarium 2005) and frequently inhabits disturbed areas (Fairly and Moore 2002). In this study, the occurrence of these species in the sites adjacent to the pine plantations suggests an ecological disturbance in those areas, as a result of the close proximity of pine plantations to native vegetation. In contrast, the vegetation at the undisturbed areas contained a greater abundance of *Persoonia acuminata, Monotoca scoparia, Clematis aristata* and *Stellaria pungens*. The species in this group are all small native shrubs, herbs and climbers (Harden 1990-1993, Fairly and Moore 2002). Their relatively lower abundance in disturbed areas suggests they are sensitive to the disturbance caused by the close proximity of pine plantations to native vegetation.

All the species discussed above are common in woodland communities across the study region (Fairly and Moore 2002, PlantNET 2005), and cannot be distinguished into two groups based on regional abundance (i.e. none of these species are uncommon on a broader scale). The species are all perennial except for *Poranthera microphylla* (which is a small annual herb (Fairly and Moore 2002), are of similar growth form (herb or small shrub), but vary in maximum height (PlantNET 2005). Future research should include manipulative experiments to contrast the growth of these potentially sensitive species between areas with and without pines and pine litter.

Clearly, our findings are correlative and further experimental work is required to link pine plantations with changes in plant species richness and abundance. However, our results do suggest that there is an edge effect associated with pine plantations. Such an edge effect may be caused by factors including altered microclimate surrounding pine plantations or the presence of pine litter that can penetrate remnant vegetation up to 50 m from adjoining plantations (Baker 2004). Pine litter may smother herbs or small shrubs, possibly explaining the reduced abundance of some such species in the disturbed sites. Plantations where pine litter dominates the forest floor have altered litter decomposition and nutrient cycling rates compared to native vegetation (Scholes and Nowicki 1998). Similar patterns may occur in native forests and woodlands where pine material also dominates the litter, which may also explain the patterns in vegetation composition we observed. Indeed, Burdon and Chilvers (1994) report that a discontinuous carpet of pine needles and shading from individual pines growing in native vegetation results in a changed environment, and ultimately changes to plant communities.

The close proximity of pine plantations to native vegetation appears to have a significant impact on composition of plant communities. It is our prediction that the patterns we observed in the vegetation assemblages are the result of the introduction of pine litter and altered ecosystem functioning. Consequently, we expect similar edge effects to occur wherever remnant vegetation abuts plantations and pine litter is exchanged.

Our novel use of PRC analysis has identified significant effects at the community level, as well as particular species that may be tolerant or sensitive to disturbance brought about by the close proximity of native vegetation to pine plantations. It is the focus of our future research to better understand both the intrinsic factors (e.g. life-history traits) and extrinsic factors (e.g. seedling growth under soils exposed to pine litter leachate) that lead to this dichotomy.

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